Crystallographic Determination of Symmetry of Aspartate Transcarbamylase

by
DON C. WILEY
WILLIAM N. LIPSCOMB
Department of Chemistry,
Harvard University,
Cambridge,
Massachusetts

Studies of trigonal and tetragonal crystalline forms of aspartate transcarbamylase show that the molecule has a three-fold and a two-fold symmetry axis.

ASPARTATE transcarbamylase (ATCase), a regulatory enzyme, catalyses the first unique reaction in the pathway of pyrimidine biosynthesis[1]. It exhibits co-operative effects[2] and is subject to feedback inhibition by cytidine triphosphate (CTP), an end product of the pathway.

Gerhart and Schachman[3] have shown that the molecule can be dissociated into two kinds of sub-units. One type binds substrate and catalyses the reaction while the other is inactive as enzyme but binds the feedback inhibitor CTP. It has further been shown that the catalytic moiety is composed of a number of identical catalytic polypeptide chains (C) and the regulatory moiety is composed of a number of identical regulatory polypeptide chains (R).

The number of copies of each protein chain and the spatial arrangement of these chains in the ATCase molecule have been studied to gain an understanding of the regulatory behaviour of the enzyme[4–6]. Previous studies on the number of binding sites for CTP and substrate[7], the amino-acid analysis and an end group analysis[8], and "fingerprint" analysis[9] of ATCase and its catalytic and regulatory chains are all consistent with a tentative model of the structure in which there are four regulatory and four catalytic chains $(RC)_4$ (ref. 4).

We report here studies of two crystalline species which require that the molecule is situated on one or another symmetry axis in different crystalline forms. Determination of the space group, water content and density of a trigonal crystal form of the CTP-ATCase complex shows that ATCase lies on a three-fold axis in the crystal. Thus the molecule has at least three-fold symmetry. Similar investigation on a tetragonal crystal form of ATCase shows that the molecule is situated on a crystallographic two-fold symmetry axis.

The first result means that the number of identical sub-units must be a multiple of three, while the second result means the number must be a multiple of two. The number of equivalent sub-units must therefore be a multiple of six. Using the molecular weights of the catalytic chain and the regulatory chain determined by Weber[6] (see preceding article) we must conclude that the number of RC pairs is six and that the molecular formula is $(RC)_6$. Furthermore, if we assume that the symmetry of the ATCase molecule is the same in the trigonal CTP-ATCase crystal as it is in the tetragonal ATCase crystal, the point group symmetry of the molecule must be D_3–32.

Properties of Crystalline Forms

Octahedral. Three crystal forms of ATCase have been prepared and studied. The first is an octahedrally shaped crystal which was obtained by dialysis from 0·04 M potassium phosphate, 2×10^{-3} M β-mercaptoethanol, and 2×10^{-4} M ethylenediamine tetraacetate at *p*H 5·9. The preparation of this crystal form has been discussed previously[8,9]. Unfortunately, X-ray patterns show that these crystals have a high degree of disorder and they have not been thoroughly characterized.

Trigonal. The second form is a trigonal crystal which was prepared in the same way except that 5×10^{-4} M CTP was added to the dialysate[9]. The space group of this form is P321 with $a = 122 \pm 0{\cdot}05$ Å and $c = 143 \pm 0{\cdot}05$ Å, and the unit cell volume is $1{\cdot}83 \times 10^6$ Å^3 (ref. 4). Crystals of this form have also been grown at *p*H 7·0 from frozen solutions of ATCase at −20° C. and from supernatants which contain the octahedral crystals and which had remained undisturbed for 3 months. The space group of this trigonal form has three special positions on which molecules could be situated. If there are three molecules per unit cell ($n = 3$) the molecule would be situated on a two-fold axis. On the other hand, two molecules ($n = 2$) per unit cell would require a molecular three-fold axis, and one molecule per unit cell ($n = 1$) would require that the molecule be at a position of 32 symmetry.

The number of molecules per unit cell can easily be calculated from the unit cell volume, molecular weight of the protein, crystal density and percentage water content of the crystal. Previously[9] we assumed a water content of 40 per cent and mistakenly calculated that N is 3, and we assigned a two-fold axis to the CTP-ATCase complex. In fact, the calculation was done incorrectly, assuming 40 per cent by weight of protein rather than the correct 40 per cent by weight of crystal.

We have now measured the water content of the trigonal crystals by the method of Low and Richards[10] in which wet crystals are weighed, dried at 100° C over P_2O_5 *in vacuo* to constant weight, and reweighed. Because our crystals are small ($0{\cdot}5 \times 0{\cdot}5 \times 0{\cdot}2$ mm), a number of them were placed in the weighing pan in order to bring the total weight to about 300 μg. Such air dried proteins commonly retain from 5 to 10 per cent of their weight in water[11], so that an additional 7·5 per cent[12] was subtracted from the apparent weight of the protein. A measurement of the salt content of the mother liquor ($0{\cdot}62 \pm 0{\cdot}02$ per cent) indicated that no correction was needed for salt which remained on the dried crystals. The results of nine measurements gave a water content of $50{\cdot}0 \pm 6{\cdot}6$ per cent, in which the error is due both to the use of small crystals, which dry rapidly during the initial weighings, and to the assumption of the amount of bound water.

The number of molecules per unit cell calculated from this value is two, and therefore the molecule must possess a three-fold axis in the trigonal crystals. If the number of molecules is taken to be either three or one, the water content would have to be 26 per cent or 74 per cent respectively, in disagreement with our measurement (Table 1).

Inasmuch as no X-ray diffraction patterns were observable from the dry crystals, these results could not be checked by that method.

Table 1. WATER CONTENT OF TRIGONAL CRYSTALS

Assumed No. of molecules per unit cell	Site symmetry of molecule	Calculated percentage of water
$n = 1$	32	74
$n = 2$	3	50
$n = 3$	2	26

The measured water content of the trigonal crystals is 50 per cent. Therefore n is 2 and the molecule has a three-fold axis in the trigonal form. The density of the crystal was assumed to be 1·10 to 1·20 g/ml.

As an independent check, however, the densities which would be expected for the trigonal crystal from the partial specific volume and number of molecules per unit cell were calculated, and the crystal density was then determined by the use of a bromobenzene-xylene gradient column[13]. The partial specific volume was calculated to be 0·737 ml./g from the specific volumes of amino-acids[14] and the amino-acid composition of ATCase[6]. Assuming that this partial specific volume is the same as that in the crystal and using the density of the buffer (1·004 g/ml.) as the density of the water in the crystal, we calculate the crystal densities which are given in Table 2, for $n = 1$, 2 or 3. The result of eight density measurements is $1{\cdot}154 \pm 0{\cdot}002$ g/ml. Comparison of this value with the calculated densities in Table 2 indicates that there must be two molecules per unit cell in the trigonal crystals, and therefore a three-fold axis in the ATCase molecule (Table 2).

Table 2. DENSITY OF TRIGONAL CRYSTALS

Assumed No. of molecules per unit cell	Calculated density* (g/ml.)
$n = 1$	1·077
$n = 2$	1·150
$n = 3$	1·223

The measured density of the trigonal crystals is $1{\cdot}154 \pm 0{\cdot}002$ g/ml. Therefore $n = 2$ and the molecule has a three-fold axis.

* The density was calculated from $\bar{V} = 0{\cdot}737$ ml./g, ϱ (buffer) = 1·004 g/ml. and V (unit cell) = $1{\cdot}83 \times 10^6$ Å^3.

Tetragonal. The third crystal form is similar to that reported by Shepherdson and Pardee[15]. It was obtained by dialysing 6 mg/ml. solutions of ATCase in 0·04 M potassium phosphate against 0·04 M potassium phosphate, 2×10^{-4} M ethylenediamine tetraacetate, 2×10^{-3} M β-mercaptoethanol, and 1·45 M $(NH_4)_2SO_4$ at *p*H 7·0. The crystals are large ($1 \times 1 \times 0{\cdot}2$ mm) square plates exhibiting birefringence through their edges but not through their flat face. The reciprocal lattice symmetry of D_{4h} and extinctions on the c* axis (l observed only for l even) establish the space group as either $P_4 22$ or $P4_2 2_1 2$. The extinction on the a* axis cannot be decided because of weakness of the pattern in this region due to the molecular transform. A four degree precession photograph of the tetragonal $0kl$ zone taken for us by Stephen Harrison with the aid of a double mirror focusing camera is shown in Fig. 1. This camera allows quantitative data collection on crystals with very large unit cells[16]. The symmetries are C_{4l} for $hk0$, C_{2l} for $0kl$, and C_{2l} for hhl zones. Unit cell dimensions are $a = 100 \pm 1$ Å and $c = 337 \pm 3$ Å. The unit cell volume is $3{\cdot}417 \times 10^6$ Å^3. In the unit cell of either of the tetragonal space groups the number of molecules could be either two, four or eight giving 222 symmetry, a two-fold axis or no symmetry, respectively.

The water content of one tetragonal crystal, measured by the method described here, yields 40 per cent. If we add 7·5 per cent for bound water, we obtain 47·5 per cent water. The salt content of the buffer was measured as $19{\cdot}0 \pm 0{\cdot}1$ per cent so that a correction was necessary for this crystalline form. Following the work on haemoglobin[17], we assume that 70 per cent of the water in the crystal is available to salt. Thus 6·3 per cent of the weight of the dry crystal is salt. The percentage of buffer by weight is therefore 53·8. Only one determination was possible because of a shortage of material. From this figure of 53·8 per cent we find that there are four molecules per unit cell. Thus ATCase has a two-fold axis in the tetragonal crystal. If there were as few as two molecules per unit cell, a water content of 75 per cent would be required (Table 3).

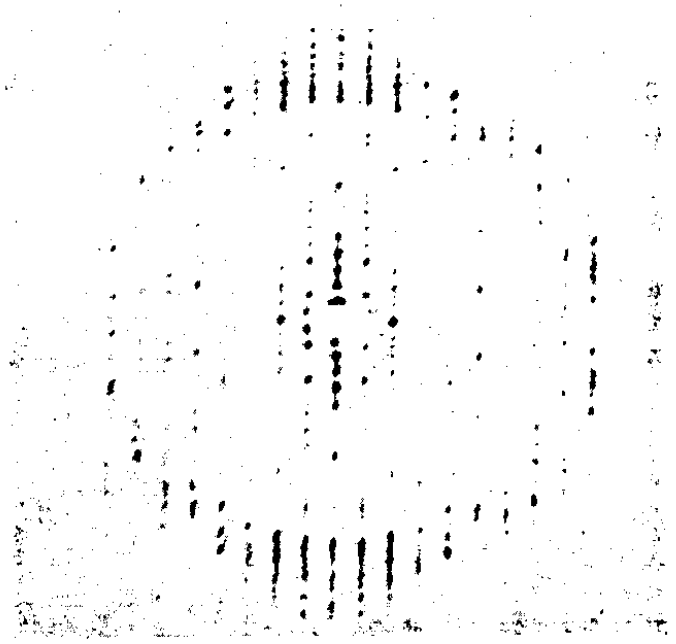


Fig. 1. Four degree precession photograph of the 0*kl* zone of a tetragonal crystal taken on a focusing camera without a layer line screen.

To corroborate this single measurement, the density of the tetragonal crystals was measured as described here. Assuming that the partial specific volume of the protein is 0·737 cc/g and that the density of the interstitial liquid in the crystal is the same as that of the mother liquor (1·112 g/ml.), we find that the calculated densities for $n=2$, $n=4$ and $n=8$ are 1·116, 1·220 and 1·330 g/ml., respectively. The result of three measurements is a density of $1{\cdot}215 \pm 0{\cdot}009$ g/ml., so that n must equal four in the tetragonal crystals. If it is assumed that only 70 per cent of the water in the crystal is accessible to salt[17], then the calculated density of the tetragonal crystals for $n=4$ is 1·202 g/ml. All of these results indicate that the ATCase molecule has a two-fold axis in the tetragonal crystal (Table 4).

Table 3. WATER CONTENT OF TETRAGONAL CRYSTALS

Assumed No. of molecules per unit cell	Site symmetry of molecule	Calculated percentage* of water
$n=2$	222	75
$n=4$	2	53
$n=8$	1	†

The measured water content of the tetragonal crystals is 53·8 per cent. Therefore n is 4 and the molecule has a two-fold axis.
* The density of the crystal was assumed to be 1·2–1·3 g/ml.
† This is an impossible case, because it requires a protein molecule smaller than ATCase.

Matthews[18] has recently tabulated results for a number of proteins, not including ATCase, giving values of V_M, the volume of the asymmetric unit per molecular weight of protein in the asymmetric unit. In our tetragonal crystal only for the case $n=4$ does the value of V_M fall into the tabulated V_M range of 1·68–3·53 Å³/dalton (Table 5).

Table 4. DENSITY OF TETRAGONAL CRYSTALS

Assumed No. of molecules per unit cell	Calculated density*
$n=2$	1·166 g/ml.
$n=4$	1·220 g/ml.
$n=8$	1·330 g/ml.

The measured density of the tetragonal crystals is $1{\cdot}215 \pm 0{\cdot}009$ g/ml. Therefore $n=4$ and the molecule has a two-fold axis.
* The density was calculated from $\bar{V}$ = 0·737 ml./g, ρ (buffer) = 1·112 g/ml., and V (unit cell) = $3{\cdot}417 \times 10^{24}$ Å³.

Table 5. TETRAGONAL CRYSTALS

Assumed No. of molecules per unit cell	Molecular weight of asymmetric unit	Values of V_M (ref. 15)
$n=2$	39,000 g/M	10·9 Å³/dalton
$n=4$	155×10^3 g/M	2·76 Å³/dalton
$n=8$	310×10^3 g/M	1·38 Å³/dalton

The volume of the asymmetric unit for these tetragonal crystals is $4{\cdot}27 \times 10^5$ Å³.

New Information about Symmetry

The number of molecules per unit cell in each of two crystal forms has been obtained from measurements of the percentage of buffer, and also independently from the density of each crystalline form. These data, when coupled with the space group of each crystal form, reveal two different symmetry axes of the ATCase molecule. These symmetry axes, in turn, restrict the number of regulatory and catalytic chains in the molecule.

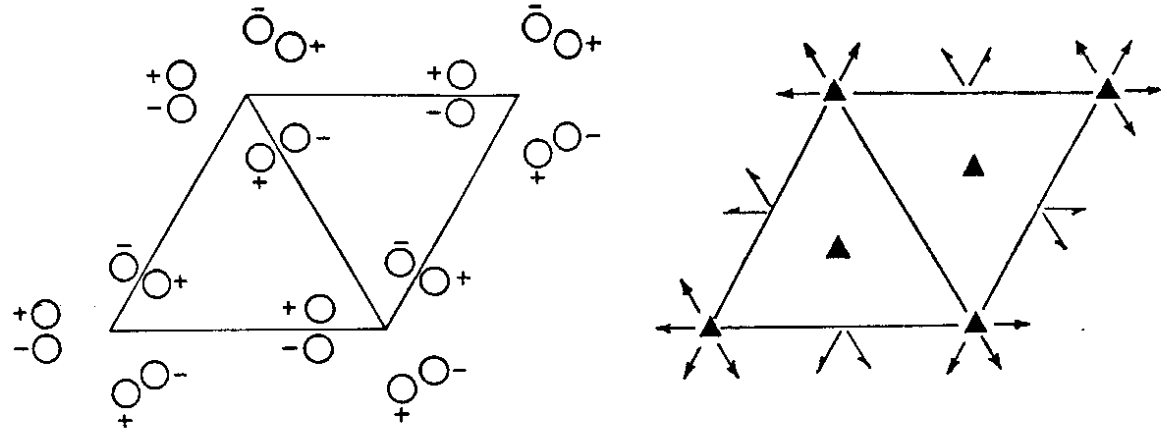
In the trigonal crystal, ATCase, when complexed with the feedback inhibitor CTP, exhibits a three-fold axis of symmetry. This axis requires the number of R chains and the number of C chains to be multiples of three. In the tetragonal crystal, ATCase alone exhibits an axis of two-fold symmetry. This two-fold axis requires the number of R chains and the number of C chains to be multiples of two. The ATCase molecule must therefore contain at least six copies of each protein chain, a result which is incompatible with earlier models[4] of ATCase.

If the molecule is composed of identical sub-units (formally RC pairs, for example), a simple way to assemble the structure is to put each sub-unit (protomer) in an identical environment. In other words, one might expect the molecule to have point group symmetry. If we assume that an ATCase molecule when removed from the trigonal crystal (which is grown in the presence of CTP, but has also been observed by us at $-20°$ C and 4° C in the absence of CTP) has the same molecular symmetry as an ATCase molecule when removed from the tetragonal crystal, then the molecule must have both three-fold and two-fold rotational symmetry. The possible point groups would be the trigonal 32, or certain point groups of higher symmetry. These point groups of higher symmetry demand at least twelve copies of each polypeptide chain. In the preceding article Weber has presented molecular weights for the R and C chains which indicate the molecule contains six copies of each chain. Thus the assumption that the ATCase molecule has the same local molecular symmetry elements in both crystals and the result (see preceding article) for the molecular weights of the R and C chains lead to point group symmetry of 32 for ATCase. This symmetry restricts the spatial arrangement of the regulatory and catalytic chains such that each of the six (RC) pairs is in an identical environment.

We thank D. L. D. Caspar for discussions and J. C. Gerhart for samples of ATCase. D. C. W. acknowledges support by a US National Science Foundation graduate fellowship. We also thank the US National Institutes of Health for support.

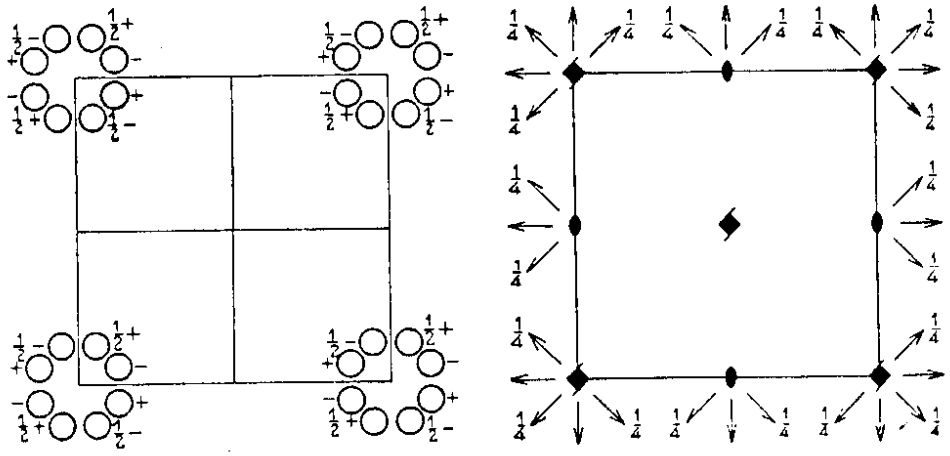
Received June 6, 1968.

[1] Yates, R. A., and Pardee, A. B., *J. Biol. Chem.*, **221**, 751 (1956).
[2] Gerhart, J. C., and Pardee, A. B., *J. Biol. Chem.*, **237**, 891 (1962).
[3] Gerhart, J. C., and Schachman, H. K., *Biochemistry*, **4**, 1054 (1965).
[4] Changeux, J. P., Gerhart, J. C., and Schachman, H. K., in *Regulation of Nucleic Acid and Protein Biosynthesis* (edit. by Konigsberger, V. V., and Bosch, L., 3440 (Biochimica and Biophysica Acta Library, **10**, Elsevier, Amsterdam).
[5] Changeux, J. P., Gerhart, J. C., and Schachman, H. K., *Biochemistry*, **7**, 531 (1968).
[6] Weber, K., *J. Biol. Chem.*, **243**, 543 (1968).
[7] Hervé, G. L., and Stark, G. R., *Biochemistry*, **6**, 374 (1967).
[8] Gerhart, J. C., and Holoubek, H., *J. Biol. Chem.*, **242**, 2886 (1967).
[9] Steitz, T. A., Wiley, D. C., and Lipscomb, W. N., *Proc. US Nat. Acad. Sci.*, **58**, 1859 (1967).
[10] Low, B. W., and Richards, F. M., *J. Amer. Chem. Soc.*, **76**, 2511 (1954).
[11] Haurowitz, F., *Chemistry and Biology of Proteins*, 91 (Academic Press, New York).
[12] North, A. T. C., *Acta Cryst.*, **12**, 512 (1959).
[13] Low, B. W., and Richards, F. M., *J. Amer. Chem. Soc.*, **74**, 1660 (1952).
[14] Schachman, H. K., in *Methods in Enzymology*, **4** (edit. by Colowick, S. P., and Kaplan, N. O.), 70 (Academic Press, New York).
[15] Shepherdson, M., and Pardee, A. B., *J. Biol. Chem.*, **235**, 3233 (1960).
[16] Harrison, S. C., *J. Appl. Crystallog.* (in the press).
[17] Perutz, M. F., *Disc. Faraday Soc.*, B, **42**, 187 (1946).
[18] Matthews, B. W., *J. Mol. Biol.*, **33**, 491 (1968).



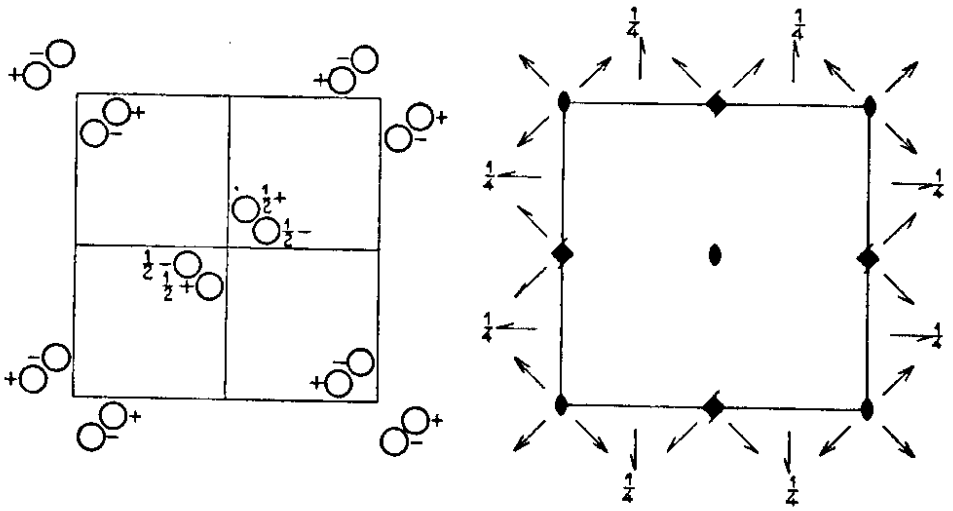
Origin at 321

Number of positions, Wyckoff notation, and point symmetry			Co-ordinates of equivalent positions	Conditions limiting possible reflections
				General:
6	*g*	1	x,y,z; $\bar{y},x-y,z$; $y-x,\bar{x},z$; $y,x,\bar{z}$; $\bar{x},y-x,\bar{z}$; $x-y,\bar{y},\bar{z}$.	No conditions
				Special:
3	*f*	2	$x,0,\frac{1}{2}$; $0,x,\frac{1}{2}$; $\bar{x},\bar{x},\frac{1}{2}$.	No conditions
3	*e*	2	$x,0,0$; $0,x,0$; $\bar{x},\bar{x},0$.	
2	*d*	3	$\frac{1}{3},\frac{2}{3},z$; $\frac{2}{3},\frac{1}{3},\bar{z}$.	
2	*c*	3	$0,0,z$; $0,0,\bar{z}$.	
1	*b*	32	$0,0,\frac{1}{2}$.	
1	*a*	32	$0,0,0$.	



Origin at 4_221

Number of positions, Wyckoff notation, and point symmetry			Co-ordinates of equivalent positions	Conditions limiting possible reflections
				General:
8	*p*	1	x,y,z; $\bar{x},\bar{y},z$; $\bar{y},x,\frac{1}{2}+z$; $y,\bar{x},\frac{1}{2}+z$; $\bar{x},y,\bar{z}$; $x,\bar{y},\bar{z}$; $y,x,\frac{1}{2}-z$; $\bar{y},\bar{x},\frac{1}{2}-z$.	*hkl*: No conditions 00*l*: $l=2n$ *h*00: No conditions
				Special: as above, plus
4	*o*	2	$x,x,\frac{3}{4}$; $\bar{x},\bar{x},\frac{3}{4}$; $\bar{x},x,\frac{1}{4}$; $x,\bar{x},\frac{1}{4}$.	0*kl*: $l=2n$
4	*n*	2	$x,x,\frac{1}{4}$; $\bar{x},\bar{x},\frac{1}{4}$; $\bar{x},x,\frac{3}{4}$; $x,\bar{x},\frac{3}{4}$.	
4	*m*	2	$x,\frac{1}{2},0$; $\bar{x},\frac{1}{2},0$; $\frac{1}{2},x,\frac{1}{2}$; $\frac{1}{2},\bar{x},\frac{1}{2}$.	*hhl*: $l=2n$
4	*l*	2	$x,0,\frac{1}{2}$; $\bar{x},0,\frac{1}{2}$; $0,x,0$; $0,\bar{x},0$.	
4	*k*	2	$x,\frac{1}{2},\frac{1}{2}$; $\bar{x},\frac{1}{2},\frac{1}{2}$; $\frac{1}{2},x,0$; $\frac{1}{2},\bar{x},0$.	
4	*j*	2	$x,0,0$; $\bar{x},0,0$; $0,x,\frac{1}{2}$; $0,\bar{x},\frac{1}{2}$.	
4	*i*	2	$0,\frac{1}{2},z$; $0,\frac{1}{2},\bar{z}$; $\frac{1}{2},0,\frac{1}{2}+z$; $\frac{1}{2},0,\frac{1}{2}-z$.	*hkl*: $h+k+l=2n$
4	*h*	2	$\frac{1}{2},\frac{1}{2},z$; $\frac{1}{2},\frac{1}{2},\bar{z}$; $\frac{1}{2},\frac{1}{2},\frac{1}{2}+z$; $\frac{1}{2},\frac{1}{2},\frac{1}{2}-z$.	
4	*g*	2	$0,0,z$; $0,0,\bar{z}$; $0,0,\frac{1}{2}+z$; $0,0,\frac{1}{2}-z$.	*hkl*: $l=2n$
2	*f*	222	$\frac{1}{2},\frac{1}{2},\frac{1}{4}$; $\frac{1}{2},\frac{1}{2},\frac{3}{4}$.	
2	*e*	222	$0,0,\frac{1}{4}$; $0,0,\frac{3}{4}$.	
2	*d*	222	$0,\frac{1}{2},\frac{1}{2}$; $\frac{1}{2},0,0$.	*hkl*: $h+k+l=2n$
2	*c*	222	$0,\frac{1}{2},0$; $\frac{1}{2},0,\frac{1}{2}$.	
2	*b*	222	$\frac{1}{2},\frac{1}{2},0$; $\frac{1}{2},\frac{1}{2},\frac{1}{2}$.	*hkl*: $l=2n$
2	*a*	222	$0,0,0$; $0,0,\frac{1}{2}$.	



Origin at 222 ([001]⟨110⟩)

Number of positions, Wyckoff notation, and point symmetry			Co-ordinates of equivalent positions	Conditions limiting possible reflections
				General:
8	*g*	1	x,y,z; $\bar{x},\bar{y},z$; $\frac{1}{2}-x,\frac{1}{2}+y,\frac{1}{2}-z$; $\frac{1}{2}+x,\frac{1}{2}-y,\frac{1}{2}-z$; $y,x,\bar{z}$; $\bar{y},\bar{x},\bar{z}$; $\frac{1}{2}-y,\frac{1}{2}+x,\frac{1}{2}+z$; $\frac{1}{2}+y,\frac{1}{2}-x,\frac{1}{2}+z$.	*hkl*: No conditions 00*l*: $l=2n$ *h*00: $h=2n$
				Special: as above, plus
4	*f*	2	$x,x,\frac{1}{2}$; $\bar{x},\bar{x},\frac{1}{2}$; $\frac{1}{2}+x,\frac{1}{2}-x,0$; $\frac{1}{2}-x,\frac{1}{2}+x,0$.	0*kl*: $k+l=2n$
4	*e*	2	$x,x,0$; $\bar{x},\bar{x},0$; $\frac{1}{2}+x,\frac{1}{2}-x,\frac{1}{2}$; $\frac{1}{2}-x,\frac{1}{2}+x,\frac{1}{2}$.	
4	*d*	2	$0,\frac{1}{2},z$; $\frac{1}{2},0,\bar{z}$; $0,\frac{1}{2},\frac{1}{2}+z$; $\frac{1}{2},0,\frac{1}{2}-z$.	*hkl*: $l=2n$ *hk*0: $h+k=2n$
4	*c*	2	$0,0,z$; $0,0,\bar{z}$; $\frac{1}{2},\frac{1}{2},\frac{1}{2}+z$; $\frac{1}{2},\frac{1}{2},\frac{1}{2}-z$.	*hkl*: $h+k+l=2n$
2	*b*	222	$0,0,\frac{1}{2}$; $\frac{1}{2},\frac{1}{2},0$.	
2	*a*	222	$0,0,0$; $\frac{1}{2},\frac{1}{2},\frac{1}{2}$.	